

## Spontaneous haploids in durum wheat: their cytogenetic characterization

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Received 6 May 2005; accepted 26 October 2005

**Key words:** Chromosome pairing, euphaploids, fluorescent genomic *in situ* hybridization (fl-GISH), *Ph1*, *Triticum turgidum*

### Summary

Durum or macaroni wheat (*Triticum turgidum* L.,  $2n = 4x = 28$ ; AABB genomes) arose as a natural hybrid between two wild species, *Aegilops speltoides* Tausch ( $2n = 2x = 14$ ; BB genome) and *Triticum urartu* Tumanian ( $2n = 2x = 14$ ; AA genome). The two progenitors hybridized in nature about half a million years ago and gave rise to tetraploid wheat, presumably in one step as a result of functioning of unreduced gametes in their hybrid BA (amphihaploid). It is easily possible to go back on the evolutionary scale and obtain durum haploids BA, and then regenerate tetraploid durum plants from them. Interestingly, such a reversion to haploidy does occur in nature as well, although at a very low frequency. This article reports on the occurrence of two spontaneous durum haploids and describes their chromosomal characteristics. The haploids (euphaploids, to be precise) had 14 somatic chromosomes, which, on fluorescent genomic *in situ* hybridization (fl-GISH), could be distinguished as 7 A-genome and 7 B-genome chromosomes. At meiosis, only 2.3 and 2.7% of the chromosomes paired in the two haploids, because of the presence of the homoeologous pairing-suppressor gene, *Ph1*. The *Ph1*-induced lack of pairing is a prerequisite for chromosome doubling through the formation of unreduced gametes that give rise to tetraploid durum wheats.

### Introduction

Durum wheat (macaroni wheat), *Triticum turgidum* L., is a natural hybrid that enjoys the benefits of hybridity and polyploidy. It is an allotetraploid ( $2n = 4x = 28$ ; AABB genomes) that resulted from hybridization between two wild species, *Aegilops speltoides* Tausch ( $2n = 2x = 14$ ; BB genome) (Sarkar & Stebbins, 1956; Wang et al., 1997) and *Triticum urartu* Tumanian ( $2n = 2x = 14$ ; AA genome) (Nishikawa, 1983; Dvořák et al., 1993). The two progenitors hybridized in nature some 500,000 years ago (Huang et al., 2002) and gave rise to tetraploid wheat, presumably in one step as a result of functioning of unreduced gametes in their hybrid BA (amphihaploid), as demonstrated in synthetic durum haploids (Jauhar et al., 2000). Thus, it is easily possible to go back on the evolutionary scale and obtain durum haploids BA, and then regenerate from them tetraploid durum plants. Interestingly, such a reversion to haploidy does occur in nature as well, although at a very low frequency. This article reports on the occurrence of two spontaneous durum haploids and describes their cytogenetic characterization.

### Materials and methods

Two haploids of durum wheat cv. Langdon were discovered in our greenhouses at the Northern Crop Science Laboratory, Fargo, North Dakota. They were first noticed as weak durum plants, and subsequent somatic chromosome counts from root tips confirmed their haploid status. Both conventional staining and

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fluorescent genomic in situ hybridization (fl-GISH) techniques (Jauhar et al., 1999; 2000) were used to study somatic and meiotic chromosomes. Fl-GISH was done on mitotic and meiotic preparations by hybridizing the A-genome chromosomes with the *Triticum urartu* genomic DNA (labeled with biotin 14-dATP, 100 ng/slide) and blocking the B-genome with *Aegilops speltoides* genomic DNA (2,000 ng/slide). The chromosome preparations were counterstained with propidium iodide (PI) and the labeled DNA was detected using fluorescein isothiocyanate (FITC). Visualizing, overlaying, and imaging were according to Jauhar et al. (2000).

### Results and discussion

Two spontaneously occurring haploids or, to be precise, euhaploids ( $2n = 2x = 14$ ) of durum wheat cv. Langdon were observed in greenhouse plantings. The haploids had diminutive characters. They had considerably reduced height, narrower leaves and thinner stems, compared to the parental cultivar they originated from. Root-tip squashes showed 14 somatic chromosomes, which on fl-GISH, could be characterized as 7 A-genome chromosomes and 7 B-genome chromosomes (Figure 1A), testifying to their euhaploid status.

The haploid plants were grown to maturity and they produced small spikes. Immature spikes in flag leaves on some tillers provided material for meiotic analyses. As expected, there was very little pairing among the 14 chromosomes (Figs. 1B, C). The two haploids had 2.3% and 2.7% of their chromosome complement paired (Table 1). The *Ph1* gene on chromosome 5B inhibits chromosome pairing among homologues, i.e., between the corresponding chromosomes of the A and B genomes (Sears & Okamoto, 1958; Riley & Chapman, 1958; Jauhar & Joppa, 1996). One dose of *Ph1* is effective in suppressing homoeologous pairing in synthetic haploids of both durum (Jauhar et al., 1999) and bread wheat (Jauhar et al., 1991). It is interesting that most spontaneous durum haploids discovered so far have very low pairing (with 2.3 to 2.7% of the chromosome complement paired) that is limited to rod bivalent formation (Table 1). The *Ph1*-induced lack of pairing is a prerequisite for the formation of unreduced gametes that give rise to tetraploid durum wheats (Jauhar et al., 2000), essentially a simulation of the event that occurred in nature (Jauhar, 2003a).

Durum wheat (AABB) is a predecessor of bread wheat (AABBDD). Having only two genomes AA

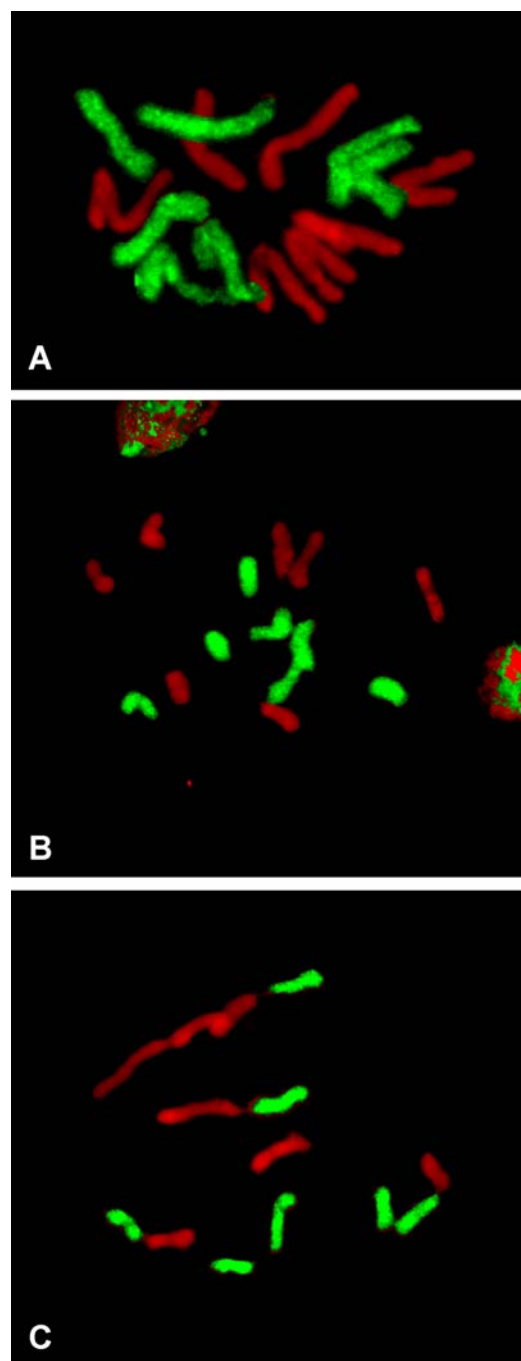


Figure 1. Somatic and meiotic chromosomes of haploids of durum wheat cv. Langdon. (A.) 14 somatic chromosomes after probing with *Triticum urartu* DNA probe. Note 7 A-genome chromosomes (green color) and 7 B-genome chromosomes (maroon color). (B.) Cell at meiotic metaphase I showing 14 univalents, 7 A-genome univalents (green color) and 7 B-genome univalents (maroon color). Note total absence of pairing in the presence of the *Ph1* gene. (C) Another cell at meiotic metaphase I showing 14 univalents, the lack of pairing caused by *Ph1*.

Table 1. Chromosome pairing in spontaneous durum haploids ( $2n = 2x = 14$ ; AB genomes)

Cultivar	No. of cells	Mean and range of chromosome configurations					Chiasma frequency:		% complement paired	Reference
		III	II			I	per cell	per II		
			Ring	Rod	Total					
Langdon	50	0.02 (0–1)	–	0.16 (0–1)	0.16 (0–1)	13.62 (12–14)	0.20 (0–2)	1.00	2.7	This study
Langdon	40	–	–	0.20 (0–1)	0.20 (0–1)	13.60 (12–14)	0.20 (0–1)	1.00	2.3	This study
Durum	94	–	–	0.37 (0–2)	0.37 (0–2)	13.19 (10–14)	0.37 (0–2)	1.00	5.3	Lacadena and Ramos (1968)
Durum	40	–	–	0.18 (0–1)	0.18 (0–1)	13.64 (12–14)	0.18 (0–1)	1.00	2.6	Kimber et al. (1978)
Santore										
Cappelli		–	–	0.17	0.17	13.56	0.17	1.00	2.4	Romero and Sendino (1982)
Bidi 17		–	–	0.05	0.05	13.90	0.05	1.00	0.7	Romero and Sendino (1982)

Note. The range of values is given in parentheses.

and BB, durum is less genetically buffered than bread wheat, and does not seem to tolerate chromosomal deficiencies very well even for single chromosomes. This is in sharp contrast to bread wheat in which a full set of monosomics has been reported (Sears, 1954). Although some monosomics have been produced in durum wheat with some difficulty, they are even more difficult to maintain (Mochizuki, 1968; Joppa & Williams, 1988). In this respect, surprisingly, there appears to be more genetic buffering in “diploid” maize than in tetraploid durum wheat; in maize, all 10 monosomics ( $2n - 1 = 19$ ) have been produced along with some occasional double monosomics ( $2n - 1 - 1 = 18$ ) and even triple monosomics ( $2n - 1 - 1 - 1 = 17$ ) (Weber, 1970; 1994). Such a chromosomal imbalance would not be tolerated by durum wheat. Haploid durum, of course, can be considered to be monosomic for all 14 chromosomes. But, then it has one full dose each of the A and B genomes. It is pertinent to state Kihara’s (1930) definition of a genome as “a set of chromosomes that form a fundamental genetic and physiological unit that is indispensable for normal development and growth of the plant”. It would appear that Kihara’s definition applies well to wheat genomes on which he spent his lifetime, but the maize genome would appear, in some ways, to defy his definition.

As eloquently stated by Magoon and Khanna (1963): “A major pathway of evolutionary advancement, perhaps to meet the rigors and diversity of environment on land, has been the assumption of dom-

inance by the diploid phase in the life cycle”. Although the haploid state of a plant does not offer any advantage from the evolutionary or survival standpoint, reversion to haploidy does nevertheless occur in several plants in nature, albeit with very low frequency. The early reports of spontaneous haploidy in durum wheat include those of Lacadena and Ramos (1968), Kimber et al. (1978), and Romero and Sendino (1982). However, genetic systems for accelerating the production of haploids have been found in several cereal crops including maize (Coe, 1959; Kermicle, 1969) and barley (Hagberg & Hagberg, 1980). Thus, Coe (1959) discovered a genetic stock (*stock 6*) of maize, which on self-pollination produces maternal haploids at the rate of about 3%. Similarly, Hagberg and Hagberg (1980) induced a mutant “factor” for haploidy initiation (*hap*) in barley. Needless to say, haploids are very important tools for basic research in cytogenetics and in several areas of biological research. They can be successfully employed in practical plant breeding for accelerated production of homozygous lines, and in genetic transformation (Jauhar & Chibbar, 1999). And efficient techniques of producing haploids are currently available in various crop plants, including durum wheat (Almouslem et al., 1998; Jauhar, 2003b).

## Acknowledgments

The author thanks Terrance Peterson and Daryl Klindworth for their assistance in this study.

## References

- Almouslem, A.B., P.P. Jauhar, T.S. Peterson, V.R. Bommineni & M.B. Rao, 1998. Haploid durum wheat production via hybridization with maize. *Crop Sci* 38: 1080–1087.
- Coe, E.H., 1959. A line of maize with high haploid frequency. *Am Nat* 93: 381–382.
- Dvořák, J., P. DiTerlizzi, H.-B. Zhang & P. Resta, 1993. The evolution of polyploid wheats: identification of the A genome donor species. *Genome* 36: 21–31.
- Hagberg, A. & G. Hagberg, 1980. High frequency of spontaneous haploids in the progeny of an induced mutation in barley. *Hereditas* 93: 341–343.
- Huang, S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill, R. Haselkorn & P. Gornicki, 2002. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proc Natl Acad Sci USA* 99: 8133–8138.
- Jauhar, P.P., 2003a. Formation of 2n gametes in durum wheat haploids: Sexual polyploidization. *Euphytica* 133: 81–94.
- Jauhar, P.P., 2003b. Haploid and doubled haploid production in durum wheat by wide hybridization. In: *Manual on Haploid and Double Haploid Production in Crop Plants*. M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Eds.). Kluwer Academic Publishers, Dordrecht, Netherlands. pp 161–167.
- Jauhar, P.P. & R.N. Chibbar, 1999. Chromosome-mediated and direct gene transfers in wheat. *Genome* 42: 570–583.
- Jauhar, P.P. & L.R. Joppa, 1996. Chromosome pairing as a tool in genome analysis: Merits and limitations. pp. 9–37. In "Methods of Genome Analysis in Plants" (P.P. Jauhar, Ed.). CRC Press, Boca Raton, Florida, USA.
- Jauhar, P.P., O. Riera-Lizarazu, W.G. Dewey, B.S. Gill, C.F. Crane & J.H. Bennett, 1991. Chromosome pairing relationships among the A, B, and D genomes of bread wheat. *Theor. Appl. Genet.* 82: 441–449.
- Jauhar, P.P., A.B. Almouslem, T.S. Peterson & L.R. Joppa, 1999. Inter- and intragenomic chromosome pairing relationships in synthetic haploids of durum wheat. *J Hered* 90: 437–445.
- Jauhar, P.P., M. Doğramacı-Altuntepe, T.S. Peterson & A.B. Almouslem, 2000. Seedset on synthetic haploids of durum wheat: Cytological and molecular investigations. *Crop Sci* 40: 1742–1749.
- Joppa, L.R. & N.D. Williams, 1988. Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. *Genome* 30: 222–228.
- Kermicle, J.L., 1969. Androgenesis conditioned by a mutation in maize. *Science* 166: 1422–1424.
- Kihara, H., 1930. Genomanalyse bei *Triticum* und *Aegilops*. II. *Cytologia* 1: 263–284.
- Kimber, G., P.J. Sallee & L.S. Bates, 1978. A polyhaploid of *Triticum turgidum*. *Cereal Res Commun* 6: 149–155.
- Lacadena, J.R., & A. Ramos, 1968. Meiotic behaviour in a haploid plant of *Triticum durum*. *Desf Genet Iber* 20: 55–71.
- Magoon, M.L. & K.R. Khanna, 1963. Haploids. *Caryologia* 16: 191–235.
- Mochizuki, A. 1968. The monosomics of durum wheat. *Proc. 3rd Int. Genet. Symp.*, pp. 310–315.
- Nishikawa, K., 1983. Species relationship of wheat and its putative ancestors as viewed from isozyme variation. *Proceedings of the 6th International Wheat Genetics Symposium, Kyoto, Japan*: pp 59–63.
- Riley, R., & V. Chapman, 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature*, 182: 713–715.
- Romero, C., & A.M. Sendino, 1982. Meiotic behaviour in haploids of *Triticum durum*. *Cereal Res Commun* 10: 191–196.
- Sarkar, P., & G.L. Stebbins, 1956. Morphological evidence concerning the origin of the B genome in wheat. *Am J Bot* 43: 297–304.
- Sears, E.R., 1954. The aneuploids of common wheat. *Missouri Agric Expt Stn Res Bull* 572.
- Sears, E.R., & M. Okamoto, 1958. Intergenomic chromosome relationships in hexaploid wheat. *Proc X Intern Cong Genet* 2: 258–259.
- Wang, G.Z., N.T. Miyashita & K. Tsunewaki, 1997. Plasmon analyses of *Triticum* (wheat) and *Aegilops*: PCR single-strand conformational polymorphism (PRC-SSCP) analyses of organellar DNAs. *Proc. Natl. Acad. Sci. USA* 94: 14570–14577.
- Weber, D.F., 1970. Doubly and triply monosomic *Zea mays*. *Maize Genet Coop Newsl* 44: 203.
- Weber, D.F., 1994. Use of maize monosomics for gene localization and dosage studies. *The Maize Handbook* (Freeling, M., and Walbot, V., Eds.). Springer-Verlag, New York, Inc., pp. 350–358.